1421

Chemistry of Some Amino Acid Derivatives of Phenoxyalkylbutyric Acids

Introduction

This paper reports on the synthesis and properties of 38 new herbicides, amino acid derivatives of 4–(2,4–dichlorophenoxy)– and 4–(2–methyl–4–chlorophenoxy)butyric acids. The biological evaluation of the compounds as selective herbicides is being conducted by other investigators and will be published later.

In the past 18 years major advances have been made in the regulation of plant growth by use of synthetic organic compounds. Many of these new synthetics have reached commercial production and are widely utilized as herbicides. The most prominent among these have been the variously substituted phenoxyalkylcarboxylic acids. The literature abounds with reports on their structural configuration in relation to mode of action, growth-regulating properties and selective herbicidal properties. These researchers were stimulated by the early work on 2,4–dichlorophenoxyacetic acid and other related compounds triggered by such preparative contributions as Pokorny's (7) in 1941. Shaw and Gentner (9) in their paper on the selective herbicidal properties of substituted phenoxyalkylcarboxylic acids have briefly reviewed the literature on some of the most significant contributions concerning the biological activity of these acids.

Preliminary investigations [2,3] have shown that the chemical attachment of an amino acid through amide linkage to variously substituted phenoxycarboxylic acids affects the selective herbicidal properties of the latter. The effect seems to depend upon the type of amino acid and its optical configuration. The optimum effect observed so far has been with an amino acid phenoxy–∝–propionic acid series.[4] Preliminary screening tests[5] (3, 4, 5) designed to measure growth-regulating activity of these compounds have been in close agreement with herbicidal evaluation data.[4] Activity tests have shown, for instance, a wide difference in the behavior pattern of compounds made from *D-*, *L-* and *DL-* optical forms of amino acids. This difference was greater in the ∝–propionic acid[4] (3) than in the

[1]Chemists, Eastern Regional Research Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Dept. of Agriculture, Phila. 18, Pa. Present address: T. F. Drake, Courtaulds, Inc., Mobile, Alabama.

[2]Gentner, W. A., and Shaw, W. C. An evaluation of several chemicals for their herbicidal properties—1957 field results. USDA Progress Report CR-25-58, ARS, Plant Industry Station, Beltsville, Maryland, January 1958, 59 p.

[3]Gentner, W. A., and Shaw, W. C. An evaluation of several chemicals for their herbicidal properties—1958 field results. USDA Progress Report CR-6-59, ARS, Plant Industry Station, Beltsville, Maryland, January 1959, 68 p.

[4]Shaw, W. C., and Gentner, W. A. Unpublished data.

[5]Krewson, C. F., Carmichael, J. F., Drake, T. F., Smale, B. C., and Mitchell, J. W. Growth Regulators. Synthesis and preliminary evaluations of amino acid derivatives of *DL*–2–(2,4,5–trichlorophenoxy)propionic acid. J. Agr. & Food Chem. In press.

acetic acid[4] (4, 5) series of phenoxy compounds tested. In most cases *D*-amino acid derivatives possessed little or no activity and *L*- and *DL*- derivatives possessed from a low to high order of activity compared with the parent acid depending upon the plant tested and the amino acid utilized.

OBJECTIVE OF THESE INVESTIGATIONS

The purpose of the studies reported herein is two-fold: (1) to create new and useful compounds from naturally occurring source materials of agricultural origin such as amino acids; and (2) through cooperative studies, to expand knowledge of the selective herbicidal properties[2,3] of the phenoxycarboxylic acids by extending the preparation of amino acid derivatives[5] (3, 4, 5, 6) to include the most important of the variously substituted phenoxybutyric acids, 4–(2,4–DB) and 4–(MCPB). This latter objective is to be accomplished by investigating the relation of structural configuration to specificity, mode of action, and herbicidal effectiveness of the compounds, for the control of weeds in a number of important crops. In this connection, Wain (10) has stated: "Reviewing the evidence at present available, it would seem that the principle underlying the use of γ–(2–methyl–4–chlorophenoxy)butyric acid, γ–(2,4–dichlorophenoxy)butyric acid, and other aryloxybutyric, caproic, and octanoic acids represents a new advance in the field of selective weed control, a development which well illustrates the value of fundamental investigations in furthering the progress of applied research."

SYNTHESIS OF AMINO ACID DERIVATIVES OF 4–(2,4–DB) AND 4–(MCPB)

For the preparation of amino acid derivatives of these phenoxy compounds procedures similar to those previously described (3, 4, 5, 6, 11) were employed. The amino acids and the two aryloxybutyric acids used in this work were the best obtainable through commercial sources.[6]

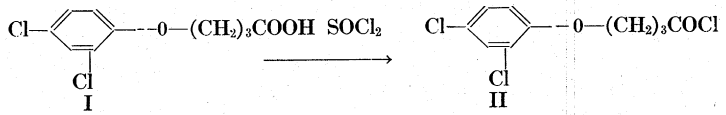
For amide coupling with the amino acids the 4–(2,4–DB) and 4–(MCPB) were first converted into their acyl chlorides (2, 5). The following description illustrates the procedure used to prepare both acyl chlorides.

4–(2,4–Dichlorophenoxy)butyryl chloride. This intermediate was prepared in 89.1% yield by the reaction of 249.1 grams (1.0 *M*) of 4–(2,4–DB) (I) with 158.0 grams (1.33 *M*) of thionyl chloride. The mixture was refluxed on a steam-bath for 4 hours, allowed to stand overnight protected from air moisture and then distilled under reduced pressure. A main fraction of 239.0 grams distilling at 136–140° C. under 0.2 mm pressure with a constant index of refraction, n^{25}_D, 1.5465 was collected following the discard of a small forerun

[6]Supplied through the countesy of Amchem Products, Inc., Ambler, Pennsylvania, and May and Baker, Ltd., Daggenham, England. Mention of company or product does not constitute endorsement by the Department over others not named.

fraction. The product (II) consisted of a colorless, supercooled liquid which crystallized upon refrigeration. The melting range of this material was 17.0–22.0° C. (uncorr.).

Analyses for $C_{10}H_9Cl_3O_2$:	Calc'd. (%)	Found (%)
Carbon	44.89	44.93
Hydrogen	3.39	3.38
Chlorine	39.76	39.43



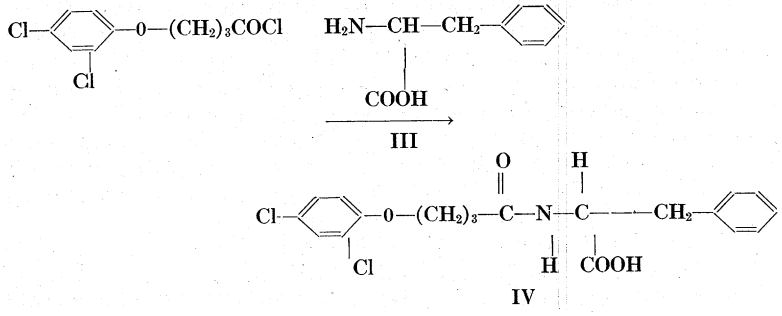
I II

4–(2–Methyl–4–chlorophenoxy)butyryl chloride. This compound was prepared in 73.5% yield using the same techniques as described above for the 4–(2,4–DB) chloride. Its boiling range was 115–122° C. at 0.2 mm with a constant index of refraction, n^{25}_D, 1.5340. The product also consisted of a colorless, supercooled liquid which crystallized when stored in the refrigerator. It melted at 5.0–9.0° C.

Analyses for $C_{11}H_{12}Cl_2O_2$:	Calc'd. (%)	Found (%)
Carbon	53.46	53.45
Hydrogen	4.90	5.19
Chlorine	28.70	28.62

N–[4–(2,4–Dichlorophenoxy)butyryl]–DL–phenylalanine. The following details are included to illustrate the general procedure used to prepare the amino acid derivatives of both 4–(2,4–DB) and 4–(MCPB): To 5.0 grams (0.03 *M*) of *DL*-phenylalanine (III) dissolved in 90 ml. of chilled 1 *N* sodium hydroxide solution was added dropwise with mechanical stirring 25 ml. of a cold (5° C.) benzene solution containing 8.0 grams of 4–(2,4–dichlorophenoxy)butyryl chloride. The addition of the benzene solution required 15–20 minutes. A temperature of 5° C. was maintained throughout the addition. Following the addition of all the benzene solution the reaction mixture, with constant stirring, was allowed to warm up to room temperature; stirring was continued for three hours.

The alkaline reaction mixture was extracted three times, once with about 50 ml. and twice with 25 ml. of diethyl ether. The combined ether extracts were washed once with about 25 ml. of distilled water, the latter being returned to the original alkaline aque-



IV

ous solution. This solution was then acidified with 1 *N* hydrochloric acid using Congo Red test paper following which it was placed in the refrigerator for an overnight period. The product (IV) was filtered off, washed with water to remove excess acid and dried to constant weight in a vacuum desiccator. The weight of the crude product was 11.0 grams (92.2% yield), m.p. 121–130° C. (Kofler micro melting point apparatus).

The crude product was dissolved in hot ethyl acetate, precipitated with petroleum ether (boiling range 63–70° C.), and allowed to remain in the refrigerator overnight. The crystals were filtered off, thoroughly washed with hot petroleum ether and dried to constant weight. This recrystallization raised the melting point to 135–139° C. The product was again recrystallized from the same solvent system. The melting point of the refined produce was 140–141° C.; it weighed 9.36 grams (78.8% yield).

Analyses for $C_{19}H_{19}Cl_2NO_4$:	Calc'd. (%)	Found (%)
Chlorine	17.89	17.68
Nitrogen	3.53	3.53

See Tables 1 and 2 for data on this and other amino acid derivatives prepared.

Discussion of Chemistry

In the preparation of most amino acid derivatives of 4–(2,4–DB) and 4–(MCPB) the crude products separated either as crystalline white solids or colorless oils which often crystallized on prolonged standing at about 5° C. Occasionally these oils did not crystallize at low temperatures. After thorough washing with water they were submitted to continuous evacuation which usually produced amorphous solids. These solids were then recrystallized in the manner previously described.

Repeated recrystallization of some derivatives was necessary to obtain satisfactory chemical and optical purity. The solvent combinations required have been indicated by footnotes in Tables 1 and 2. In these refinements of crude amino acid derivatives no attempts were made to improve yields by working up mother liquors from the recrystallizations.

Although one or more attempts were made to prepare *D-*, *L-*, and *DL-* aspartic acid derivatives of 4–(2,4–DB) and 4–(MCPB) only one was successful, that of *DL*-aspartic acid with 4–(MCPB). This same experience has been encountered in attempting to prepare the three aspartic acid derivatives of *DL*–2–(2,4,5–trichlorophenoxy)propionic acid[5]: only the *DL*-aspartic acid derivative was successfully isolated. However, this was not found to be the case with aspartic acid derivatives of *DL*–2–(2,4–dichlorophenoxy)propionic acid or aspartic acid derivatives of chlorine-substituted phenoxyacetic acids (3, 4, 5, 11); all three optical forms of aspartic acid derivatives of these acids were successfully prepared.

Difficulty was encountered in synthesizing both *D-* and *L*-threonine derivatives of 4–(2,4–DB) and 4–(MCPB). Repeated unsuccessful attempts were made using Schotten-Baumann amide coupling

Table 1. Yields, physical properties and analyses of amino acid derivatives of 4–(2,4-dichlorophenoxy) butyric acid.

N–[4–(2,4–Dichlorophenoxy) butyryl]–	M.P., °C[a] (Corr.)	Yield per cent		Formula	Analyses[b]					
					Cl per cent		N. per cent		Optical Rotation	
		Crude	Refined		Calcd.	Found	Calcd.	Found	$[\alpha]_D^{25}$	C (g./100 ml.) in pyridine
L–alanine	98.0–100.0[c]	76.4	56.2	$C_{13}H_{15}Cl_2NO_4$	22.14	21.85	4.37	4.35	− 7.83 ±0.4	2.00
D–alanine	96.0– 98.0	51.2	41.8		22.14	21.70	4.37	4.41	+ 8.36 ±0.4	2.00
DL–alanine	116.0–119.0	48.6	40.1		22.14	21.80	4.37	4.40		
L–leucine	82.0– 84.0	70.1	48.8	$C_{16}H_{21}Cl_2NO_4$	19.57	19.36	3.87	3.85	−14.40 ±0.4	2.00
D–leucine	71.0– 75.0[d]	81.0	34.9		19.57	19.52	3.87	3.84	+12.81 ±0.6	2.00
DL–leucine	103.0–104.0	84.7	63.6		19.57	19.09	3.87	3.92		
L–methionine	55.0– 60.0	77.2	17.8	$C_{15}H_{19}Cl_2NO_4S$	18.65	18.40	3.68	3.70	− 3.54 ±0.5	2.00
D–methionine[e]	123.0–125.0[f]	92.6	15.0		18.65	18.98	3.68	3.65	− 0.1 ±0.6	2.05
DL–methionine	122.0–123.0	85.0	66.3		18.65	18.48	3.68	3.68		
L–phenylalanine	132.5–134.0[g]	87.5	71.3	$C_{19}H_{19}Cl_2NO_4$	17.89	17.75	3.53	3.61	+16.23 ±0.6	2.05
D–phenylalanine	131.0–132.0	70.9	60.6		17.89	17.79	3.53	3.65	−18.00 ±3.0	0.65
DL–phenylalanine	140.0–141.0	92.2	78.8		17.89	17.68	3.53	3.53		
L–threonine[e]	99.0–101.0[h]	53.0	10.6	$C_{14}H_{17}Cl_2NO_5$	20.25	20.43	4.00	3.95	+ 2.48 ±0.8	0.93
D–threonine[e]	89.0– 95.0	—	4.3		20.25	20.56	4.00	3.86	− 1.63 ±0.4	2.00
DL–threonine[e]	168.0–169.0[g]	57.1	30.9		20.25	20.42	4.00	4.09		
L–tryptophane	185.0–186.5	73.5	71.3	$C_{21}H_{20}Cl_2N_2O_4$	15.95	15.85	6.47	6.38	+20.67 ±0.5	2.00
D–tryptophane	187.0–189.0[i]	88.0	74.3		15.95	16.09	6.47	6.45	−21.14 ±0.5	2.00
DL–tryptophane	168.0–169.0[c]	85.0	61.9		15.95	15.97	6.47	6.42		
N,N′–bis–[4–(2,4–Dichlorophenoxy) butyryl]— *L*–cystine	154.0–155.5[c,j]	43.4	27.8	$C_{26}H_{28}Cl_4N_2O_8S_2$	20.19	19.73	3.99	3.98	64.68 ±0.6	2.05

[a]Recrystallized one or more times from ethyl acetate–petroleum ether unless otherwise indicated.
[b]Analyses by J. S. Ard and E. L. Nutter.
[c]And recrystallized from hot ethanol-water.
[d]And purified through preparation of its cyclohexylamine salt.
[e]Prepared by J. F. Carmichael.
[f]Recrystallized from hot ethanol–n–hexane, hot dilute acetic acid, acetone–water, dioxane–water and dichloroethane–n–hexane.
[g]Recrystallized from hot ethanol–water only.
[h]And from hot dilute acetic acid.
[i]Recrystallized from hot methanol–water (norit treated) and hot ethanol–water.
[j]Also extracted 12 hours with petroleum ether in a Soxhlet.

Table 2. Yields, physical properties and analyses of amino acid derivatives of 4–(2–methyl–4–chlorophenoxy) butyric acid.

N–[4–(2–Methyl–4–chlorophenoxy) butyrl]—	M.P., °C.[a] (Corr.)	Yield, per cent		Formula	Analyses[b]					
					Cl per cent		N per cent		Optical Rotation	
		Crude	Refined		Calcd.	Found	Calcd.	Found	$[\alpha]_D^{25}$	C (g./100 ml.) in pyridine
L–alanine	108.0–110.0[c]	84.9	53.1	$C_{14}H_{18}ClNO_4$	11.83	12.00	4.67	4.46	− 4.84 ±0.4	2.00
D–alanine	113.0–115.0	76.8	49.0		11.83	11.76	4.67	4.64	+ 5.16 ±0.4	2.00
DL–alanine	118.0–119.0	79.5	43.5		11.83	11.85	4.67	4.68		
DL–aspartic acid	148.0–151.0	44.3	22.4	$C_{15}H_{18}ClNO_6$	10.31	10.20	4.07	4.14		
L–leucine	71.0–73.0	71.7	41.1	$C_{17}H_{24}ClNO_4$	10.37	10.35	4.10	4.10	− 7.38 ±0.4	2.00
D–leucine	89.0–90.0	84.8	73.1		10.37	10.40	4.10	4.11	+11.15 ±0.4	2.00
DL–leucine	111.0–112.0	69.3	43.8		10.37	10.53	4.10	4.12		
L–methionine	70.0– 73.0	84.3	65.8	$C_{16}H_{22}ClNO_4S$	9.85	10.04	3.89	3.81	− 1.54 ±0.4	2.00
D–methionine	67.0– 70.0[d]	77.1	26.3		9.85	9.75	3.89	3.87	+ 0.72 ±0.5	2.00
DL–methionine	99.0–100.0	75.2	46.4		9.85	10.07	3.89	3.86		
L–phenylalanine	134.0–135.0	58.5	55.9	$C_{20}H_{22}ClNO_4$	9.43	9.67	3.73	3.69	+20.30 ±0.4	2.00
D–phenylalanine	130.0–132.0	90.4	81.0		9.43	9.55	3.73	3.68	−23.03 ±0.4	2.00
DL–phenylalanine	135.0–137.0	98.4	90.4		9.43	9.50	3.73	3.68		
L–threonine (−H_2O)	158.0–159.0[e]	—	4.5	$C_{15}H_{18}ClNO_4$	11.37	11.29	4.49	4.51	0.00 ±0.4	2.00
D–threonine (−H_2O)	160.0–161.0[e]		9.0		11.37	11.26	4.49	4.50	0.00 ±0.4	2.00
DL–threonine	170.0–172.0	83.0	52.2	$C_{15}H_{20}ClNO_5$	10.75	10.78	4.24	4.23		
L–tryptophane	187.0–189.0[f]	94.6	65.8	$C_{23}H_{23}ClN_2O_4$	8.50	8.47	6.72	6.73	+21.65 ±0.5	2.00
D–tryptophane	190.5–192.0[f]	—	78.2		8.50	8.54	6.72	6.79	− 22.13 ±0.5	2.01
DL–tryptophane	172.0–173.0[f]	96.6	80.6		8.50	8.52	6.72	6.78		

[a]Recrystallized one or more times from ethyl acetate–petroleum ether unless otherwise stated.
[b]Analyses by J. S. Ard and E. L. Nutter.
[c]Preceded by recrystallization from ethanol–water.
[d]And purified through preparation of its cyclohexylamine salt.
[e]Preceded by recrystallization from chloroform–petroleum ether.
[f]From methanol–water only. Methanol solution of *L*–tryptophane derivative norit treated.

techniques described for IV above. Finally, by this method small amounts of the *D*- and *L*-threonine derivatives of 4–(2,4–DB) were obtained after failures with the Ronwin method (8), the Carter et al. method (1) and a number of variations on these procedures. It was first thought that the *L*-threonine derivative of 4–(2,4–DB) was a lactone due to the close check of an elemental chlorine analysis with the calculated value for a lactone. The compound therefore was supplied at this stage of purification for biological evaluation along with the other members of these two series. However, nitrogen analysis performed at a later date failed to confirm lactone formation. Further purification gave the desired product, giving the data presented in Table 1, which are for the highly purified product. Biological evaluations made on the impure compound will be interpreted in the light of the presence of a small amount of the phenoxy parent acid contaminant.

The preparation and identification of the *D*- and *L*-threonine derivatives of 4–(MCPB) will be the subject of another report since this chemistry became somewhat involved. The chlorine and nitrogen analyses data support the formation of the desired compounds but with one less molecule of water. This water was formed by the loss of the hydroxyl group of threonine and a hydrogen atom from its terminal methyl group resulting in the formation of a terminal double bond:

$$R{-}CHOH{-}CH_3 \xrightarrow{-H_2O} R{-}CH{=}CH_2.$$

Since the completion of these studies threonine derivatives of phenoxy acids in general have been found to possess moderate water solubility which may account for the low yields experienced when Schotten-Baumann procedures have been used.

SUMMARY

1. Thirty-eight new amino acid derivatives, 19 of 4–(2,4–DB) and 19 of 4–(MCPB) have been synthesized and their properties studied.

2. Both 4–(2,4–DB) and 4–(MCPB) formed amides with the *D*-, *L*-, and *DL*- optical forms of alanine, leucine, methionine, phenylalanine, threonine, and tryptophane.

3. Unsuccessful attempts were made to prepare the optically active aspartic acid derivatives of both 4–(2,4–DB) and 4–(MCPB). *DL*-asparatic acid did form an amide with 4–(MCPB but not with 4–(2,-4–DB).

4. *D*- and *L*-Threonine formed unique compounds with 4–(MCPB) involving the terminal methyl group of threonine in the formation of a double bond.

5. Most of these amino acid derivatives of the phenoxybutyric acids were readily prepared and analyzed and possessed sharp melting points; hence they may be useful in the characterization of amino acids.

6. Biological evaluation of these compounds as selective herbicides is being conducted by other investigators and will be reported later.

Some of the compounds are also being evaluated as estrogens, fungicides, insecticides, and anticancer agents.

LITERATURE CITED

1. CARTER, H. E., HANDLER, P., and STEVENS, C. M. Azlactones. III. Acylation of amino acids in pyridine. Jour. Biol. Chem. 138:619–626. 1941.
2. FREED, V. H. Preparation and reactions of 2,4–dichlorophenoxyacetyl chloride. Jour. Amer. Chem. Soc. 68:2112. 1946.
3. KREWSON, C. F., DRAKE, T. F., MITCHELL, J. W., and PRESTON, W. H., JR. Plant growth regulators. Preliminary screening tests of amino acid derivatives of 2–(2′,4′–dichlorophenoxy)propionic acid. J. Agr. & Food Chem. 4:690–693. 1956.
4. ———, ———, NEUFELD, C. H. H., FONTAINE, T. D., MITCHELL, J. W., and PRESTON, W. H., JR. Growth regulators. Amino acid derivatives of 4–chlorophenoxyacetic acid and their plant-regulating effects in preliminary screening tests. J. Agr. & Food Chem. 4:140–143. 1956.
5. ———, NEUFELD, C. H. H., DRAKE, T. F., FONTAINE, T. D., MITCHELL, J. W., and PRESTON, W. H., JR. Synthetic plant-growth modifiers. IV. 2–Methyl–4–chlorophenoxyacetyl derivatives of amino acids. Weeds 3:28–37. 1954.
6. ———, SAGGESE, E. J., CARMICHAEL, J. F., ARD, J. S., DRAKE, T. F., MITCHELL, J. W., and SMALE, B. C. Growth regulators. Synthesis and preliminary evaluations of amide, lactic acid, and terpenoid derivatives of substituted phenoxycarboxylic acids. J. Agr. & Food Chem. 7:118–122. 1959.
7. POKORNY, R. Some chlorophenoxyacetic acids. Jour. Amer. Chem. Soc. 63:1768. 1941.
8. RONWIN, E. Direct acetylation of α–amino acids and of α–hydroxy acid derivatives. Jour. Org. Chem. 18:127–132. 1953.
9. SHAW, W. C., and GENTNER, W. A. The selective herbicidal properties of several variously substituted phenoxyalkylcarboxylic acids. Weeds 5:75–92. 1957.
10. WAIN, R. L. Herbicidal selectivity through specific action of plants on compounds applied. J. Agr. & Food Chem. 3:128–130. 1955.
11. WOOD, J. W., and FONTAINE, T. D. Synthetic plant-growth regulators. III. 2,4–Dichlorophenoxyacetyl derivatives of amino acids. Jour. Org. Chem 17:891–896. 1952.